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AFTER MHC-MATCHED ALLOGRAFTING, HOST-DERIVED LANGERHANS CELLS (LCS) PERSIST IN SKIN AND CUTANEOUS LYMPH NODES IN THE STEADY-STATE AND ARE THE TARGETS OF DLI-MEDIATED ALLORESPONSE

Durakovic, N.; Bezak, K.B.; Skarica, M.; Fuchs, E.J.; Luznik, L. Sidney Kimmel Comprehensive Cancer Center at Johns Hopkins, Baltimore, MD.

The mechanisms that govern T cell-mediated alloresponses in the post-transplant setting, particularly T cell-dendritic cell (DC) interactions, are critical for developing strategies to augment their antitumor effects and limit toxicity such as GVHD. We hypothesize that kinetics of DC reconstitution and the number of host DCs persisting in various lymphoid tissues after alloBMT may differ and if characterized, may be relevant in guiding T cell alloresponses following transplant. To analyze DC reconstitution in the MHC-matched setting, we used a B6Ly 5.2 ($H2^b$; CD45.1+)→C3H.SW ($H2^b$; CD45.2+) model that differs in expression of the CD45 marker on host and donor hematopoietic tissues. We found that the donor to host DC turnover is rapid with near full conversion to donor DC chimerism in the spleen and mesenteric lymph nodes but not in the cutaneous lymph nodes (CLNs). The dominant residual host DC population in LNs is characterized by the low expression of CD8+ but high expression of DEC-205 and gp40 (Ep-CAM), the profile completely consistent with the phenotype of LCs. We reproducibly detected residual host-derived LCs in the chimeras CLNs for at least 6 months after alloBMT and found them to constitutively express an activated phenotype. Levels of expression of the costimulatory molecules, CD80, CD40, and especially CD86 on host-derived LCs was actually higher in comparison to donor-derived LCs. The skin origin of host-derived DCs in LNs was confirmed by analysis of CD11c+ that migrate out of ex vivo cultured epidermal sheets. Because the majority of the CD11c+ was of host origin, this suggests that alloresponse against minor histocompatibility antigens is not sufficient enough to cause LC replacement. The persistence of residual host LCs after alloBMT prompted us to determine whether they serve as the target of the DLI-mediated alloresponse. Administration of DLI 3 weeks after conditioning resulted in the decrease of residual host-derived LCs in the CLNs and recruitment of donor derived LCs to the skin. Our data suggest the following: (a) After myeloablative conditioning and transplantation of MHC-matched marrow, there is a rapid replacement of secondary lymphoid organs with donor marrow-derived DCs but that the significant amount of skin DCs are of host origin; (b) during steady-state conditions after alloBMT, host-derived LCs bearing an activated phenotype are continuously present in the CLNs; and (c) host-derived LCs are the targets of the DLI-mediated alloresponse.

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IN VITRO RAPAMYCIN AND COSTIMULATION GENERATES TH1/TC1 OR TH2/TC2 CENTRAL MEMORY EFFECTORS: DIFFERENTIAL REGULATION BY IN VIVO RAPAMYCIN AFTER ALLOGENEIC BMT

Fowler, D.H.; Foley, J.; Jung, U. Experimental Transplantation and Immunology Branch, NCI, Bethesda, MD.

Rapamycin (rapa) inhibition of GVHD is associated with Th2/Tc2 polarization. We evaluated (a) whether rapa directly regulates cytokine polarity independent of antigen-presenting-cells (APC) and (b) whether rapa modulates effector Th1/Tc1 and Th2/Tc2 responses. To evaluate aim (a), T cells were costimulated in IL-4 or IL-12 containing media. Surprisingly, Th1/Tc1 differentiation proceeded in rapa (10 micromolar; 10-fold expansion over 6 days). IL-2 and IFN-g secretion was preserved with no increase in IL-4, IL-5, IL-10, or IL-13; fas ligand cytolysis was modestly reduced. Th2/Tc2 differentiation also proceeded in rapa, with similar 10-fold expansion, maintenance of type II cytokines, and partial preservation of granule cytolysis. Rapa-generated Th1, Tc1, Th2, and Tc2 cells were CD44+CD62L+, had increased expansion to alloantigen (B6-into-CB6F1 BMT), and maintained cytokine polarity in vivo. Rapa thus does not directly modulate cytokine polarity and can be used to generate Th1/Tc1 and Th2/Tc2 cells of central memory phenotype that possess enhanced capacity for T1 or T2 polarization post-BMT. To evaluate aim (b), rapa-generated T cells were studied after allogeneic BMT with or without rapa therapy. Congenic donor Th1/Tc1 cells (CD45.1) and Th2/Tc2 cells (CD90.1) were used in cell-mixing experiments to evaluate effector T-cell cross-regulation. Rapa-generated Th2/Tc2 cells potently inhibited Th1/Tc1 cell number and cytokine secretion; this Th2/Tc2 shift was fully preserved with rapa therapy. Rapa-generated Th1/Tc1 cells potently inhibited Th2/Tc2 cell number and function post-BMT; however, this Th1/Tc1 shift was completely abrogated by rapa therapy (> 90% reduction in Th1/Tc1 number and function [IL-2 and IFN-g secretion]. Given the inhibitory role of rapa on APC function, we hypothesized that rapa polarizes cytokine production through APC modulation. To evaluate this hypothesis, CB6F1 dendritic cells (DCs) were generated in vitro. Such F1 DCs were immune competent, because their adoptive transfer after syngeneic BMT and Th1/Tc1 cell transfer induced graft-versus-host reaction (allospecific Th1/Tc1 cell IFN-g secretion). However, transfer of F1 DC in the setting of rapamycin therapy did not yield allospecific Th1/Tc1 cell responses post-BMT. In conclusion, the Th2/Tc2 polarization associated with rapa therapy is mediated indirectly, most likely through APC modulation that preferentially inhibits Th1/Tc1 cells.

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ALLOGENEIC B CELL RESPONSE TO H-Y MINOR HISTOCOMPATIBILITY ANTIGENS AFTER DONOR LYMPHOCYTE INFUSION CORRELATES WITH DISEASE RESPONSE

Miklos, D.B.[1]; Miller, K.[1]; Kim, H.T.[2]; Lee, S.J.[2]; Alyea, E.P.[2]; Cutler, C.[2]; Antin, J.H.[2]; Soiffer, R.J.[2]; Ritz, J.[2] 1. Stanford University Medical Center, Stanford, CA; 2. Dana-Farber Cancer Institute, Boston, MA.

Donor lymphocyte infusion (DLI) can induce remission in many patients who relapse after allogeneic hematopoietic stem cell transplantation (HSCT). We have previously demonstrated that male HSCT patients with female donors frequently develop high-titer antibody responses to H-Y antigens that correlate with disease remission. We sought to determine whether allogeneic B-cell responses develop after DLI. We expressed 5 recombinant H-Y proteins (DBY, UTY, ZFY, RPS4Y, and EIF1AY) and developed sensitive ELISA to quantify the development of specific anti-HY antibodies. First, we studied prophylactic DLI. Twenty-six patients who received T-cell-depleted HSCT followed 5–7 months later by prophylactic CD8 depleted DLI were tested for H-Y antibodies pre-DLI and 6–12 months after DLI. No H-Y antibodies were detected in any of the pre-DLI serum samples. However, all 6 male HSCT patients with female donors (F→M HSCT) developed high-titer antibodies against at least 1 H-Y antigen after DLI. In contrast, only 1/20 of the other donor/recipient gender combinations (4 M→M, 8 F→F, 8 M→F) resulted in H-Y antibody ($P < .005$). Thus, mHA disparity is required for the development of allogeneic B cell responses after DLI. This robust development of H-Y antibody in 6/6 F→M patients who received TCD transplantation and prophylactic DLI was significantly greater than in the 3/9 who developed H-Y antibodies after receiving the same TCD HSCT without DLI ($P = .03$). This suggests that DLI augments allogeneic B-cell responses after T-cell-depleted HSCT. To examine the effects of therapeutic DLI, we studied 24 F→M HSCT patients who relapsed 60 days to 15 years (median 704 days) after transplant and subsequently received either unmanipulated DLI or CD8-depleted DLI. Only 2/24 had any H-Y antibody at the time of relapse. After DLI, 17/24 (71%) developed antibody to at least 1 H-Y antigen, and this correlated with complete remission after DLI ($P < .001$). Disease progression continued in all 7 patients who did not develop H-Y antibodies, but 15 of 17 patients who developed H-Y antibodies also attained complete remission. H-Y antibodies developed rapidly and were detected as early as 26 days after DLI. Complete remission was attained with similar frequencies after both CD8 depleted DLI (7/12) and unmanipulated DLI (8/12). In summary, H-Y antibodies frequently develop in male patients after infusion of female donor lymphocytes, and this allogeneic B cell response correlates with clinical response to DLI.

H-Y Antibody Results in Male Patients with Female Donors

	Any H-Y Antibody	2 or more H-Y Antibodies
TCD HSCT + prophylactic CD8 depleted DLI	6/6 100%	4/6 67%
TCD HSCT alone	3/9 33%	1/9 11%
CD8 depleted DLI for relapse	9/12 75%	9/12 75%
Unmanipulated DLI for relapse	8/12 67%	2/12 17%

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IN VIVO TRAFFICKING OF CD4+CD25+ REGULATORY T-CELLS IN ALLOGENEIC RECIPIENTS USING BIOLUMINESCENCE IMAGING

Nguyen, V.H.; Wieland, C.; Contag, C.; Negrin, R. Stanford University School of Medicine, Stanford, CA.

CD4+CD25+ regulatory T-cells (Treg) have the potential to suppress aberrant immune responses and to regulate peripheral T-cell homeostasis. In a murine allogeneic bone marrow transplantation (BMT) model, we previously showed that Treg suppress graft-versus-host-disease (GVHD) without abrogating the beneficial graft-versus-tumor immunological effect. In the current study, we investigate the in vivo trafficking of Treg to better understand how localization may affect their regulatory function. We have developed and characterized a transgenic mouse which constitutively expresses the luciferase gene in all hematologic cells. Treg from the spleen and lymph nodes of luc+ transgenic FVB/N (H-2^q) mice were cotransplanted into lethally-irradiated Balb/c (H-2^d) host along with wild-type FVB/N T-cell-depleted bone marrow (TCD-BM) cells and whole splenocytes, the latter containing approximately 30% T cells, which induce GVHD. Bioluminescence imaging (BLI) was performed at various time points. Within the first 48 hours, Treg localized to the cervical lymph nodes (LN) and the spleen. By day 3, signal is detected in other LN (axillary, mesenteric, inguinal) as well as Peyer's patches and liver. Signal intensity, measured by photons/second/mouse, significantly increased and peaked on day 4, consistent with the migration and proliferation of Treg to and at these secondary lymphoid organs, respectively. Skin infiltration of Treg is noted on day 6, congruent with a decreased intensity in the spleen, liver, and lymph nodes. A similar pattern of early migration and proliferation of effector immune cells is noted in the GVHD control group, which is transplanted with wild-type FVB/N TCD-BM and luc+ FVB/N whole splenocytes. However, with the GVHD group, the signal intensity continues to increase at all sites. Continued BLI of the Treg group up to day 45 demonstrates persistent strong signal in lymphoid organs and skin sites. Clinically, the Treg group had no significant evidence of GVHD. Chimerism studies on day 45 show complete donor origin, however, lymphoid reconstitution of CD4+ and CD8+ T cells is delayed in the GVHD control group and enhanced in the recipients transplanted with Treg. The aforementioned results indicate that in vivo, Treg proliferate and survive long-term. In addition, they colocalize with effector immune cells to secondary lymphoid tissues to positively impact clinical outcomes and lymphoid reconstitution following major MHC-mismatched BMT.

HEMATOPOIESIS/MESENCHYMAL CELLS

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BONE MARROW-DERIVED MESENCHYMAL STEM CELLS AUGMENT TISSUE ENGINEERED ENTEROCYTES

Gandia, C.; Yu, H.; Santiago, S.; Coats, E.; Defaria, W.; Ruiz, P.; Tzakis, A.; Kleiner, G.I. University of Miami School of Medicine, Miami, FL.

Bone marrow-derived MSCs have been demonstrated to have multipotential differentiating ability. Despite recent advances in in vitro models, a reliable method of expansion and long-term maintenance of normal intestinal epithelial cells (IECs) is lacking. Most of the limited data arise from studies based on T84 and Caco-2 colon carcinoma lines. We evaluated a novel protocol of isolation and long-term culture of IEC from rodent enterocytes. The ability of MSC to augment tissue engineered small bowel was evaluated. **Methods:** Small intestine from neonatal DA rats (12 days old) was harvested and digested using cold and warm solution of dispase/collagenase. Primary culture cells in minimal media with high glutamine were initiated. No growth factors were used. Confirmation of epithelial and endothelial cells in the primary culture was verified by Immunohistochemical markers (cytokeratin 19, 5, and 8; anti-cytokeratin+ basal monoclonal antibody; MadCAM-1; and antilaminin B2). Long-term cultures (> 100 days) were obtained. Cytokine secretion panels were determined by BioPlex assay and intestinal metabolites (citrulline, praline, glutamine, lactate) by GC/mass spectroscopy. Bone marrow-derived MSCs were added to cultures. **Results:** Primary culture tissue expressed intestinal epithelial and endothelial. Cytokeratin 19 was the most abundant, followed by cytokeratin 5 and 8, MadCAM-1, anticytokeratin+ basal monoclonal antibody, and laminin B12 (in descending order). Primary and long-term (> 100 days) cultures secreted citrulline, a specific marker of functional enterocytes. Addition of bone marrow-derived MSCs significantly augmented citrulline production ($P < .05$). Cytokines TNF alpha, IL-10, and GMCSF correlated with citrulline levels. The results suggest that bone marrow-derived MSCs augment intestinal development in an in vitro rodent model and may be useful in tissue engineering applications.

HISTOCOMPATIBILITY/ALTERNATIVE STEM CELL SOURCES

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PARTICULAR HLA-DPB1 ALLELE MISMATCHES PREDICT FOR WORSE OVERALL SURVIVAL IN RECIPIENTS OF UNRELATED DONOR HAEMATOPOIETIC STEM CELL TRANSPLANTS

Shaw, B.E.; Marsh, S.G.E.; Mayor, N.P.; Madrigal, A. The Anthony Nolan Research Institute, Royal Free Hospital, Hampstead, London, United Kingdom.

In the HLA-DPB1 molecule there are 6 hypervariable regions (HVR), A to F, in exon 2, which encode for the ββ domain and form the peptide binding groove (PB). Within each of these HVRs are 1 or more amino acids that are polymorphic, and this polymorphism is important in determining the peptides that will bind the recognition by T-cell receptors or both. Mismatches at this level may predict for transplant complications, and this analysis may contribute to a better understanding of the function of DPB1. We analyzed the outcome in 282 mixed transplant pairs who received an hematopoietic stem cell transplant (HSCT) from an unrelated donor. All of the pairs were matched at the allelic level for class I and II. Transplant pairs were assessed as matched or mismatched for HVRs A to F, in a graft-versus-host direction. Mismatches at both amino acid position 65 (within HVR D) and 57 (within HVR C) were associated with transplant complications. Position 65 was matched in 231 (82%) pairs and mismatched in 51 (18%). Matched pairs had a significantly improved overall survival (OS) compared with mismatched pairs (49% vs 35%; log rank $P = .039$). Position 57 was matched in 233 pairs (83%) and mismatched in 49 (17%). There was no significant association between matching at either position and graft-versus-host disease or disease relapse. In contrast, mismatched pairs had a significantly increased rate of transplant-related mortality (TRM). In conclusion, we have shown a significantly worse OS, largely mediated by an increased TRM, in recipients of HSCT from UD which are mismatched at amino acid positions 57 and 65 of the DPB1 molecule.